IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

Application of: Y.V.S.N. MURTHY	Confirmation No.: 4452
Application No.: 10/623,114	Group Art Unit: 1617
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For: COMPOSITIONS CONTAINING PRODRUGS OF FLORFENICOL	Attorney Docket No.: 13390-07011

DECLARATION UNDER 37 C.F.R. § 1.132

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Sir:

I, Yerramilli V.S.N. Murthy, an inventor on the above-identified U.S. patent application, *i.e.*, U.S. patent application serial no. 10/623,114 ("the '114 application"), published as US 2005/0014828 ("the '828 publication"), hereby declare that:

1. I am a citizen of the United States and I reside at 2212 Oak Stream Lane, Apex North Caroline, 27523.

2. I am a research manager for IDEXX laboratories Inc. I have a Ph.D. in chemistry and have been involved in chemical research for over 15 years. I am an inventor on over 20 U.S. patents or patent applications.

3. Under my direction and supervision a study was conducted to compare the pharmacokinetics in calves of sub-cutaneously administered florfenicol (Nufluor®, commercially available from Schering-Plough Corp. of Kenilworth, NJ), sub-cutaneously administered florfenicol acetate, sub-cutaneously administered florfenicol butyrate, and sub-cutaneously administered florfenicol hexanoate.

4. Florfenicol butyrate was formulated at 350 mg/ml in 10% propylene glycol ("florfenicol butyrate composition") accoding to the following general procedure: weigh about 70 g of florfenicol butyrate into a 200 mL volumetric flask, add about 20 mL of propylene glycol, fill the flask to a volume of about 100 mL with glycerol formal to provide a suspension, sonicate the suspension for about 15 minutes followed by shaking for about 1 hour on a shaker, add more glycerol formal to provide a volume of about 150 mL, shake the resulting mixture until a clear solution is obtained, fill the flask to 200 mL with glycerol formal, and mix it well to provide a homogenous solution.

5. A similar procedure was used to formulate florfenicol acetate and florfenicol hexanoate at 350 mg/ml in 10% propylene glycol ("florfenicol acetate composition" and florfenicol hexanoate composition," respectively).

6. The pharmacokinetic study involved:

$ administering the florfenicol butyrate composition to each of four calves by intramuscular injection at a dosage of 40 mg/kg,

$ administering the florfenicol acetate composition to each of two calves by intramuscular injection at a dosage of 40 mg/kg,

$ administering the florfenicol hexanoate composition to each of two calves by intramuscular injection at a dosage of 40 mg/kg,

$ administering commercially available Nufluor® to each of two calves by intramuscular injection at a dosage of 20 mg/kg as per label,

and analyzing the concentration of florfenicol in the serum of each calf as a function of time as described in the '828 publication (*See*, the '828 publication at ¶ [0054]). Injections were administered to the muscles of the neck or buttocks as described in the '828 publication (*See*, the '828 publication at ¶ [0054]).

7. The results of the pharmacokinetic study are depicted graphically in FIG. 1. Each data point for florfenicol butyrate represent the average serum concentration of the four calves that were injected. Similarly, each data point for florfenicol acetate, florfenicol hexanoate, and florfenicol (*i.e.*, Nufluor®) represent the average serum concentration of the two calves that were injected.

8. FIG. 1 graphically depicts that the florfenicol butyrate composition provides a therapeutically effective serum concentration of florfenicol (*i.e.*, greater than 1 μg/mL) for longer than any of the other formulations. Only florfenicol butyrate provides a serum concentration of florfenicol that is above 1 μg/mL for more than about 36 hours. To be therapeutically effective it is necessary to maintain a serum concentration of florfenicol that is above 1 μg/mL. Thus, when administering florfenicol, florfenicol acetate, or florfenicol hexanoate, it is necessary to adminster multiple injections to maintain a therapeutically effective serum concentration of florfenicol for a time period sufficient to treat an infection in an animal.[1] In contrast, a single injection of florfenicol butyrate, by providing a therapeutically effective serum concentration of florfenicol for more than 36 hours (in fact, a serum concentration of florfenicol above 1 μg/mL for three (3) days), maintains a therapeutically effective serum concentration of florfenicol for a time period that is sufficient to treat an infection without requiring multiple injections. Avoiding

[1] Indeed, we note that the labeling for Nuflor® indicates that it "should be administered by intramuscular injection to cattle at a dose rate of 20 mg/kg body weight (3 mL/100 lbs). A second dose should be administered 48 hours later. Alternatively, NUFLOR Injectable Solution can be administered by a single subcutaneous injection to cattle at a dose rate of 40 mg/kg body weight (6mL/100 lbs). Do not administer more than 10 mL at each site" (*See*, prescribing information for Nuflor®, attached hereto as Exhibit A)

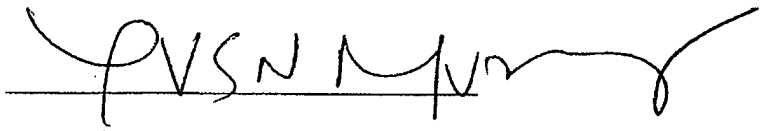
multiple injections is advantageous in that it is safer, simpler, less stressful for the animal, and more cost effective.

9. Furthermore, administering florfenicol acetate avoids the initial rapid increase (*i.e.*, "spike") in florfenicol serum concentration that is observed when Nufluor® is administered to a calf. Avoiding this "spike" in florfenicol serum concentration provides a safer phatmacokinetic profile and is believed to minimize some of the adverse effects that can be associated with administering Nufluor®. The results of these experiments demonstrate that administering florfenicol butyrate results in a pharmacokinetic profile for serum florfenicol concentration that is superior to the pharmacokinetic profile obtained when florfenicol (*i.e.*, Nufluor®) or other florfenicol esters are administered.

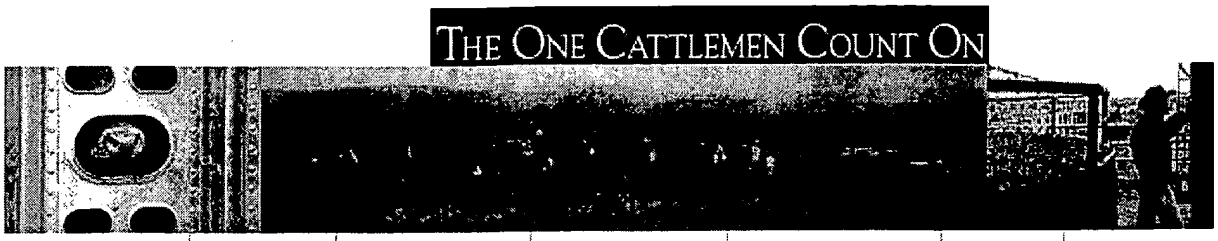
10. Only florfenicol butyrate shows the above described advantageous pharmacokinetic profile. Ester prodrugs of florfenicol wherein the hydrocarbon chain of the ester functional group is shorter than that of florfenicol butyrate (*i.e.*, florfenicol acetate) or longer than that of florfenicol butyrate (*i.e.*, florfenicol hexanoate) do not show the advantageous pharmacokinetic profile. Without wishing to be bound by theory, I believe that the unique pharmacokinetic profile of florfenicol butyrate is due to a combination of factors present only in a florfenicol butyrate composition. Specifically, I believe the advantageous pharmacokinetic profile is the result of florfenicol butyrate having a specific solubility in water (and, thus, a unique release rate when administered to an animal, such as a calf) and a unique metabolism rate by esterases. The combination of these two factors results in a unique composition that, when administered to an animal, such as a calf, provides a serum level of florfenicol that is unexpectedly safe and effective.

7. I further declare that all statements made herein of my knowledge are true and all statements made on information and belief are believed to be true, and further that these statements are made with the knowledge that willful false statements and the like so made are punishable by fine or imprisonment, or both, under section 1001 of Title 18 of the United States Code and that such willful false statements may jeopardize the validity of this application or any patent issuing thereon.

Dated this 31st day of JAN, 2008



Yerramilli V.S.N. Murthy, Ph.D.



Nuflor® (FLORFENICOL)

Product Disclosure

Second Title

F-19027139
NADA #141-063, Approved by FDA

Product Information

Nuflor®
(FLORFENICOL)

Injectable Solution 300 mg/mL

For Intramuscular and Subcutaneous Use in Cattle Only.

CAUTION: Federal law restricts this drug to use by or on the order of a licensed veterinarian.

DESCRIPTION: NUFLOR Injectable is a solution of the synthetic antibiotic florfenicol. Each milliliter of sterile NUFLOR Injectable Solution contains 300 mg of florfenicol, 250 mg n-methyl-2-pyrrolidone, 150 mg propylene glycol, and polyethylene glycol q.s.

CLINICAL PHARMACOLOGY: The pharmacokinetic disposition of NUFLOR Injectable Solution was evaluated in feeder calves following single intramuscular administration at the recommended dose of 20 mg/kg. NUFLOR Injectable Solution was also administered intravenously to the same cattle in order to calculate the volume of distribution, clearance, and percent bioavailability[1] (Table 1).

TABLE 1. Pharmacokinetic Parameter Values for Florfenicol Following IM Administration of 20 mg/kg Body Weight to Feeder Calves (n=10).

Parameter	Median	Range
C_{MAX} (µg/mL)	3.07*	1.43 - 5.60
T_{MAX} (hr)	3.33	0.75 - 8.00
$T_{1/2}$ (hr)	18.3**	8.30 - 44.0
AUC (µg•min/mL)	4242	3200 - 6250
Bioavailability (%)	78.5	59.3 - 106
Vd_{SS} (L/kg)***	0.77	0.68 - 0.85
Cl_T (mL/min/kg)***	3.75	3.17 - 4.31

* harmonic mean
** mean value
*** following I.V. administration
C_{MAX} Maximum serum concentration
T_{MAX} Time at which C_{MAX} is observed
$T_{1/2}$ Biological half-life
AUC Area under the curve
Vd_{SS} Volume of distribution at steady state
Cl_T Total body clearance

Florfenicol was detectable in the serum of most animals through 60 hours after intramuscular administration with a mean concentration of 0.19 µg/mL. The protein binding of florfenicol was 12.7%, 13.2%, and 18.3% at serum concentrations of 0.5, 3.0, and 16.0 µg/mL, respectively.

MICROBIOLOGY: Florfenicol is a synthetic, broad-spectrum antibiotic active against many gram-negative and gram-positive bacteria isolated from domestic animals. It is primarily bacteriostatic and acts by binding to the 50S ribosomal subunit and inhibiting bacterial protein synthesis. *In vitro* and *in vivo* activity has been demonstrated against commonly isolated bacterial pathogens involved in bovine respiratory disease (BRD) including *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*, as well as against commonly isolated bacterial pathogens involved in bovine interdigital phlegmon including *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*.

The minimum inhibitory concentrations (MICs) of florfenicol for BRD organisms were determined using isolates obtained from natural infections from 1990 to 1993. The MICs for interdigital phlegmon organisms were determined using isolates obtained from natural infections from 1973 to 1997 (Table 2).

TABLE 2. MIC Values* of Florfenicol Against Bacterial Isolates From Natural Infection of Cattle.

Organism	Isolate Numbers	MIC_{50}** (µg/mL)	MIC_{90}** (µg/mL)
Pasteurella haemolytica	398	0.50	1.00
Pasteurella multocida	350	0.50	0.50
Haemophilus somnus	66	0.25	0.50
Fusobacterium necrophorum	33	0.25	0.25
Bacteroides melaninogenicus	20	0.25	0.25

*The correlation between the *in vitro* susceptibility data (MIC values) and clinical response has not been confirmed.

**The minimum inhibitory concentration for 50% and 90% of the isolates.

INDICATIONS: NUFLOR Injectable Solution is indicated for

treatment of bovine respiratory disease (BRD), associated with *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*, and for the treatment of bovine interdigital phlegmon (foot rot, acute interdigital necrobacillosis, infectious pododermatitis) associated with *Fusobacterium necrophorum* and *Bacteroides melaninogenicus*. Also, it is indicated for the control of respiratory disease in cattle at high risk of developing BRD associated with *Pasteurella haemolytica*, *Pasteurella multocida*, and *Haemophilus somnus*.

RESIDUE WARNINGS: Animals intended for human consumption must not be slaughtered within 28 days of the last intramuscular treatment. Animals intended for human consumption must not be slaughtered within 38 days of subcutaneous treatment. Do not use in female dairy cattle 20 months of age or older. Use of florfenicol in this class of cattle may cause milk residues. A withdrawal period has not been established in preruminating calves. Do not use in calves to be processed for veal.

WARNINGS: NOT FOR HUMAN USE. KEEP OUT OF REACH OF CHILDREN. This product contains materials that can be irritating to skin and eyes. Avoid direct contact with skin, eyes, and clothing. In case of accidental eye exposure, flush with water for 15 minutes. In case of accidental skin exposure, wash with soap and water. Remove contaminated clothing. Consult a physician if irritation persists. Accidental injection of this product may cause local irritation. Consult a physician immediately. The Material Safety Data Sheet (MSDS) contains more detailed occupational safety information.

For customer service, adverse effects reporting, and/or a copy of the MSDS, call 1-800-211-3573.

CAUTION: Not for use in cattle of breeding age. The effects of florfenicol on bovine reproductive performance, pregnancy, and lactation have not been determined. Intramuscular injection may result in local tissue reaction which persists beyond 28 days. This may result in trim loss of edible tissue at slaughter. Tissue reaction at injection sites other than the neck is likely to be more severe.

ADVERSE EFFECTS: Inappetence, decreased water consumption, or diarrhea may occur transiently following treatment.

TOXICOLOGY: A 10X safety study was conducted in feeder calves. Two intramuscular injections of 200 mg/kg were administered at a 48-

hour interval. The calves were monitored for 14 days after the second dose. Marked anorexia, decreased water consumption, decreased body weight, and increased serum enzymes were observed following dose administration. These effects resolved by the end of the study.

A 1X, 3X and 5X (20, 60, and 100 mg/kg) safety study was conducted in feeder calves for 3X the duration of treatment (6 injections at 48-hour intervals). Slight decrease in feed and water consumption was observed in the 1X dose group. Decreased feed and water consumption, body weight, urine pH, and increased serum enzymes, were observed in the 3X and 5X dose groups. Depression, soft stool consistency, and dehydration were also observed in some animals (most frequently at the 3X and 5X dose levels), primarily near the end of dosing.

A 43-day controlled study was conducted in healthy cattle to evaluate effects of NUFLOR Injectable Solution administered at the recommended dose on feed consumption. Although a transient decrease in feed consumption was observed, NUFLOR Injectable Solution administration had no long-term effect on body weight, rate of gain, or feed consumption.

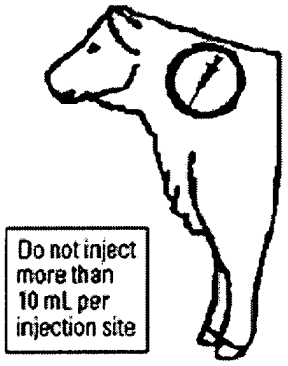
DOSAGE AND ADMINISTRATION: For treatment of bovine respiratory disease (BRD) and bovine interdigital phlegmon (foot rot): NUFLOR Injectable Solution should be administered by intramuscular injection to cattle at a dose rate of 20 mg/kg body weight (3 mL/100 lbs). A second dose should be administered 48 hours later. Alternatively, NUFLOR Injectable Solution can be administered by a single subcutaneous injection to cattle at a dose rate of 40 mg/kg body weight (6mL/100 lbs). Do not administer more than 10 mL at each site. **The injection should be given only in the neck.**

NOTE: Intramuscular injection may result in local tissue reaction which persists beyond 28 days. This may result in trim loss of edible tissue at slaughter. Tissue reaction at injection sites other than the neck is likely to be more severe.

For control of respiratory disease in cattle at high-risk of developing BRD: NUFLOR Injectable Solution should be administered by a single subcutaneous injection to cattle at a dose rate of 40 mg/kg body weight (6 mL/100 lbs). Do not administer more than 10 mL at each site. **The injection should be given only in the neck.**

NUFLOR DOSAGE GUIDE		
ANIMAL WEIGHT (lbs)	IM NUFLOR DOSAGE 3.0 mL/100 lb Body Weight (mL)	SC NUFLOR DOSAGE 6.0 mL/100 lb Body Weight (mL)
100	3.0	6.0
200	6.0	12.0
300	9.0	18.0
400	12.0	24.0
500	15.0	30.0
600	18.0	36.0
700	21.0	42.0
800	24.0	48.0
900	27.0	54.0
1000	30.0	60.0

Recommended Injection Location



Clinical improvement should be evident in most treated subjects within 24 hours of initiation of treatment. If a positive response is not noted within 72 hours of initiation of treatment, the diagnosis should be reevaluated.

STORAGE CONDITIONS: Store between 2°-30°C (36°-86°F). Refrigeration is not required. The solution is light yellow to straw colored. Color does not affect potency.

HOW SUPPLIED: NUFLOR Injectable Solution is packaged in 100 mL (NDC 0061-1116-04), 250 mL (NDC 0061-1116-05), and 500 mL (NDC 0061-1116-06) glass sterile multiple-dose vials.

REFERENCE: 1. Lobell RD, Varma KJ, et al. Pharmacokinetics of florfenicol following intravenous and intramuscular doses to cattle.

J Vet Pharmacol Therap. 1994; 17:253-258.

January 1999
Mfd. for Schering-Plough Animal Health Corp.,
Union, NJ 07083
by Merial Limited, Iselin, NJ 08830-3077.

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TAKE TIME

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